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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 31/19

A1

(11) International Publication Number: WO 92/16200

(43) International Publication Date: 1 October 1992 (01.10.92)

(21) International Application Number: PCT/US92/02078

(22) International Filing Date: 20 March 1992 (20.03.92)

(30) Priority data:
672,577 20 March 1991 (20.03.91) US

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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THE USE OF HYDROXAMIC ACID DERIVATIVES TO INHIBIT VIRAL REPLICATION

(57) Abstract

Hydroxamic acid derivatives such as deferoxamine (a drug that is already approved by the Food and Drug Administration for treating iron toxicity in humans) are useful for the inhibition of HIV and other viruses.

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THE USE OF HYDROXAMIC ACID DERIVATIVES TO INHIBIT VIRAL REPLICATION

TECHNICAL FIELD

The present invention relates to an antiviral compound that inhibits viral replication, a pharmaceutical composition containing the compound and a method of using the compound to inhibit viral replication.

BACKGROUND OF THE INVENTION

Despite considerable research, very few drugs have been discovered that are useful in the treatment of viral infections. Most of the drugs that are useful are nucleoside analogues. At present, the only approved treatment for human immunodeficiency virus (HIV) infection is azidothymidine (AZT), a drug with substantial toxicity and less than optimal efficacy. Other drugs under study include dideoxyinosine (ddI) and dideoxycytosine (ddC). There is no approved drug for the treatment of any other human retroviral infection.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a method for inhibiting the growth of a virus by use of a compound that is low in toxicity, but which also exhibits a significant anti-viral effect. Specifically, the present invention is directed to a method for inhibiting the growth of a virus, preferably a virus that is dependent on reverse transcriptase for replication, and method which comprises providing to a cell infected with the virus an effective viral growth inhibiting amount of a hydroxamic acid derivative having antiviral activity or a physiologically acceptable salt thereof or a physiologically acceptable chelate thereof. The hydroxamic acid derivative can be provided to cells growing in vitro that are infected by a virus or can be

administered to a human (or animal) infected by a virus. It is also possible that the hydroxamic acid derivative could be administered to a human (or animal) who is at high risk of being exposed to a virus in order to prevent viral replication upon such exposure.

In the treatment of individuals infected with HIV, the hydroxamic acid derivative can be administered to the human as soon as such a diagnosis has been made (such as by a positive immune response to HIV) or it can be administered after symptoms of the infection have appeared, i.e., after the patient has symptoms of Acquired Immunodeficiency Syndrome (AIDS) or AIDS-Related Complex (ARC).

DETAILED DESCRIPTION OF THE INVENTION

The hydroxamic acid derivatives that are useful in accordance with this invention include, but are not limited to, deferoxamine (also called desferrioxamine) (N-[5-[3-[(5-aminopentyl)-hydroxycarbamoyl]propionamido]-pentyl]-3-[[5-(N-hydroxyacetamido)-pentyl]carbamoyl]propiono-hydroxamic acid); salicylhydroxamic acid; hexanohydroxamic acid; octanohydroxamic acid; decanohydroxamic acid; dodecanohydroxamic acid; nicotinohydroxamic acid; o-aminobenzohydroxamic acid; rhodotorulic acid; and cholylhydroxamic acid; or a physiologically acceptable salt thereof or a physiologically acceptable chelate thereof. A preferred hydroxamic acid derivative is deferoxamine (DFX) which, when administered to a patient, is preferably administered in the form of a physiologically acceptable salt such as deferoxamine mesylate (Ciba-Geigy).

DFX is an iron-chelating compound that has recently been shown to have a significant inhibitory effect on the

growth in vitro of cell lines created from human hepatocellular carcinoma (HCC) (Hann et al, Hepatology, 11:566-569 (1990); Tabor et al, J. of Medical Virology (1991) (in press)), human neuroblastoma (Blatt et al, Cancer Research, 49:2925-2927 (1989)), human lymphoma (Becton et al, Cancer Research, 49:4809-4812 (1989)), and human leukemia (Becton et al, Cancer Research, 49:4809-4812 (1989)).

The antiviral activity of hydroxamic acid derivatives, of which DFX is an example, against HIV-1, strain HTLV-III_B in vitro (in H9 cells) has been established. Hydroxamic acid derivatives such as DFX may be active against a number of different viruses in vitro and in vivo and in particular it can be expected that hydroxamic acid derivatives such as DFX may be active in vitro and in vivo against any virus whose replication is dependent on reverse transcriptase including HIV such as HIV-1 and HIV-2 and at least all members of the retrovirus family such as human T-lymphotropic virus (HTLV) including HTLV-I (the causative agent for adult T-cell leukemia/lymphoma and related syndromes) and HTLV-II. Hepatitis B virus also uses an unusual reverse transcriptase mechanism of replication and the drug should be effective against it as well.

Hydroxamic acid derivatives may also have antiviral activity against other viruses which do not utilize reverse transcriptase. DFX has been shown to have a relatively low level of cytotoxicity against non-cancerous cells. For example, DFX is not usually toxic in humans treated for iron toxicity and is not toxic against H9 cells as apparent from the studies reported herein. However, DFX does inhibit growth of cancer cells but it should not be characterized as cytotoxic.

DFX can form a chelate with iron or with a number of other metal ions or other cations. This happens after it has been administered to a patient, and this is a goal of the currently approved use of DFX. It is possible that DFX or other hydroxamic acid derivatives could be administered for antiviral purposes as a chelate. It is possible that the action as an antiviral occurs either in the form administered or after it has formed a chelate.

Hydroxamic acid derivatives could be used for the treatment and/or prophylaxis of human and animal viral diseases, particularly mammalian diseases, caused by the above-mentioned viruses and possibly other viruses. It is contemplated that the hydroxamic acid derivative will be formulated into a pharmaceutical composition comprising an effective antiviral amount of the hydroxamic acid derivative or physiologically acceptable salt or chelate thereof and a pharmaceutically acceptable carrier. For intravenous administration, the hydroxamic acid derivative could be administered without a carrier. An effective antiviral amount of the pharmaceutical composition will be administered to the subject, human, animal or mammal, in a manner and dose that inhibit or prevent viral replication. The amount of the hydroxamic acid derivative or physiologically acceptable salt or chelate thereof and the specific pharmaceutically acceptable carrier will vary depending upon the mode of administration and the type of viral condition being treated.

The routes of administration should be intravenous (i.v.), intraperitoneal (i.p.), or intramuscular (i.m.), subcutaneous (s.c.), or intradermal (i.d.), with i.v. and i.m. being preferred. The compound could be administered orally (p.o.) when it has been made in an appropriate

form for oral administration.

For localized virus infections, the pharmaceutical compositions may be administered topically as an ointment, cream, aerosol, or powder, or given as eye or nose drops, etc.

It can also be administered as a suppository.

In a particular aspect the pharmaceutical composition comprises the hydroxamic acid derivative or a physiologically acceptable salt or chelate thereof in effective unit dosage form. As used herein the term "effective unit dosage" or "effective unit dose" is denoted to mean a predetermined antiviral amount sufficient to be effective against the viruses in vivo. Pharmaceutically acceptable carriers are materials useful for the purpose of administering the compound, which are preferably non-toxic, and may be solid, liquid or gaseous materials, which are otherwise inert and medically acceptable and are compatible with the active ingredients. Preservatives may also be included in the formulation. The pharmaceutical compositions may be formulated with one active ingredient (the hydroxamic acid derivative or physiologically acceptable salt or chelate thereof) or in combination with other active ingredients such as other antiviral agents.

The compositions of DFX may contain 0.1%-99% by weight of the active material. For i.v. administration the preferred concentration is 0.1% to 25% weight/volume (w/v). For other parenteral routes, the preferred concentration is 0.1% to 50% w/v.

For oral administration, fine powders or granules may contain diluting, dispersing and/or surface active agents, and may be presented in a draught, in water or in a syrup; in capsules in the dry state or in a non-aqueous

solution or suspension, wherein suspending agents may be included; in tablets, wherein binders and lubricants may be included; in caplets; in micronized "sprinkle" form; or in a suspension in water or a syrup. Where desirable or necessary, flavoring, preserving, suspending, thickening or emulsifying agents may be included. Tablets and granules may be coated. For buccal administration the compositions may take the form of tablets or lozenges formulated in a conventional manner.

For administration as drops, as for eye infections, the compounds may be presented in aqueous solution in a concentration of from about 0.1 to 30%, more preferably 0.5 to 2.0%, most preferably 0.5% to 1.5% w/v. The solution may contain antioxidants, buffers, preservatives, etc.

The compounds according to the invention may also be formulated for injection and may be presented in unit dose form in ampoules or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for reconstitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds may be included in an aerosol or mist that is inhaled by a patient having a pulmonary infection or a systemic viral infection.

The compounds may be administered by intrathecal administration for treatment of a central nervous system (CNS) HIV or HTLV infection or other viral infections of the CNS.

The compounds may be applied into any body orifice such as the nose, oral cavity and ears, in the form of a spray or drops. They may be applied into body orifices in the form of a suppository or cream.

For systemic administration the daily dosage as employed for adult or pediatric human treatment will range from 0.1-200 mg/kg/day, preferably 1 to 10 mg/kg/day, which may be administered in 1 to 6 daily doses, for example, depending on the route of administration and the condition of the patient. When the compositions comprise dosage units, each unit will preferably contain 2 mg to 100 mg of active ingredient. For serious infections the compound may be administered by intravenous infusion using, for example, 0.01 to 10 mg/kg/hr of the active ingredient (the i.v. administration not to exceed 15 mg/kg/hr).

In yet a further aspect of the invention there is provided a method of treating or preventing viral infections in animals (particularly mammals) or humans, which comprises the administration of an effective antiviral amount, as hereinbefore defined, of a hydroxamic acid derivative or a physiologically acceptable salt or physiologically acceptable chelate thereof.

In yet a further aspect of the invention, there is provided a pharmaceutical composition in unit dosage form wherein each unit dose contains 1 to 250 mg of active ingredient, preferably 2-100 mg of active ingredient. For example, 1 to 250 mg of the active ingredient can be placed in a sterile container such as a vial together with a pharmaceutically acceptable injectable diluent.

The compound should be administered in an amount calculated to produce a blood level of at least 30 μM,

preferably 30 to 60 μM, for a period of one to thirty days or longer. The compound of the present invention can be administered to the patient either alone or in combination with other antiviral compounds such as AZT. When given in combination with other antiviral compounds, a lower blood level may be effective.

EXAMPLE

Duplicate cultures of H9 cells (5×10^5 cells/ml) infected with human immunodeficiency virus type 1 (HIV-1) (strain HTLV-III_B) (10^4 infectious units/ml) were maintained for 7 days in each of five coded media preparations, as shown in Table I. Cultures were split 1:2 at day 3. At day 7, coded samples of supernates were tested for HIV p24 antigen using a commercial capture enzyme immunoassay (Coulter Immunology, Hialeah, FL); coded samples of DNA extracted from cell lysates were tested for HIV proviral DNA by polymerase chain reaction using primer pairs derived from the gag and env regions of the genome (Hewlett et al, J. AIDS, 3:714-720 (1990)).

In these blinded studies, DFX inhibited the expression of p24 antigen and significantly reduced the detectable levels of gag and env genes in H9 cell cultures after seven days. The inhibition was dose-dependent (as shown in Table I); 30 μM DFX had the same effect on p24 expression as 187 μM azidothymidine (AZT) (Boehringer-Mannheim) (50 μg/ml). Cultures grown in medium lacking DFX and AZT produced substantial concentrations of p24, and the signals for gag and env sequences were strongly positive. Viability of the H9 cells was >70% at day 7 in cultures grown in DFX and AZT, as well as in the control cultures. Three independent experiments were conducted with similar results for p24 expression. Evaluation of gag and env were available

only in one experiment. Data provided in Table I are from the two experiments conducted under code.

The mechanism of this inhibition is unknown. DFX has been shown to inhibit DNA synthesis in seven human cancer cell lines from three different organ systems (reviewed in Tabor et al, J. of Medical Virology (1991) (in press)). In PHA-stimulated lymphocytes, inhibition by DFX of DNA synthesis has been reported to be due to the inhibition of iron-dependent ribonucleotide reductase (Hoffbrand et al, British Journal of Haematology, 33:517-526 (1976)). DFX could have inhibited HIV-1 by interfering with the RNA-dependent DNA synthesis that occurs early in each infectious cycle.

The 30 μM concentration of DFX is equivalent to the blood level theoretically reached with an intravenous dose of 99 mg in a human with a 5-liter blood volume, well below the maximum recommended dose for DFX in humans, 2.0 g i.v. DFX has been administered experimentally to nine adults at much higher doses, 150 mg/kg/day for five days, without recognized adverse reactions (Donfrancesco et al, Cancer Research, 50:4929-4930 (1990)).

The observation of in vitro inhibition of HIV-1 by DFX reported here may suggest a new mechanism of viral inhibition.

Table I

Medium Containing	Experiment A p24*	gag*	env*	Cell Viability*	Exp. B. p24*	Cell Viability*
30 μM DFX***	90	+	-	80%	0	95%
20 μM DFX	230	++	+	82%	0	70%
10 μM DFX	1174	++	+	75%	370	74%
187 μM AZT	117	-	-	78%	0	86%
Distilled H_2O**	1174	++	+	87%	400	79%

* p24 (pg/ml) by capture enzyme immunoassay of supernate; gag and env in DNA extracted from cell lysates and analyzed by polymerase chain reaction, scored visually on an autoradiogram on a scale from - to +++ by comparison with reference standards (Hewlett et al, J. AIDS, 3:714-720 (1990)); cell viability determined by trypan blue exclusion.

** Distilled water was added to the control medium in the same volume (0.25%) as the deferoxamine was added to create a 30 μM solution.

*** In this Example, DFX was in the form of deferoxamine mesylate.

CLAIMS:

1. Use of a hydroxamic acid derivative having antiviral activity or a physiologically acceptable salt thereof or a physiologically acceptable chelate thereof for the inhibition of the growth of a virus wherein an effective viral growth inhibiting amount of said hydroxamic acid derivative is provided to a cell infected with said virus.

2. Use according to claim 1, wherein said hydroxamic acid derivative is selected from the group consisting of deferoxamine; salicylhydroxamic acid; hexanohydroxamic acid; octanohydroxamic acid; decanohydroxamic acid; dodecanohydroxamic acid; nicotinohydroxamic acid; o-aminobenzohydroxamic acid; rhodotorulic acid; cholylhydroxamic acid; or a physiologically acceptable salt thereof or a physiologically acceptable chelate thereof.

3. Use according to claim 1, wherein deferoxamine mesylate is contacted with cells in vitro.

4. Use according to claim 1, wherein deferoxamine mesylate is administered to a human.

5. Use according to claim 1, wherein said virus is one that is dependent on reverse transcriptase for replication.

6. Use according to claim 1, wherein said virus is one that is not dependent on reverse transcriptase for replication.

7. Use according to claim 1, wherein deferoxamine mesylate is administered to a human infected with a virus that is dependent on reverse transcriptase for replication.

8. Use according to claim 1, wherein deferoxamine mesylate is administered to a human infected with a virus

that is not dependent on reverse transcriptase for replication.

9. Use according to claim 4, wherein said human is infected with human immunodeficiency virus.

10. Use according to claim 9, wherein said human has acquired immunodeficiency syndrome or AIDS-related complex.

11. Use according to claim 4, wherein said human is infected with hepatitis B virus.

12. Use according to claim 4, wherein said human is infected with HTLV.

13. Use according to claim 1, wherein said hydroxamic acid derivative or physiologically acceptable salt or physiologically acceptable chelate thereof is administered to an animal infected with a virus that is dependent on reverse transcriptase for replication.

14. Use according to claim 1, wherein said hydroxamic acid derivative or physiologically acceptable salt or physiologically acceptable chelate thereof is administered to an animal infected with a virus that is not dependent on reverse transcriptase for replication.

15. Use of a hydroxamic acid derivative or a physiologically acceptable salt or a physiologically acceptable chelate thereof for the prevention of viral replication wherein said hydroxamic acid derivative is administered to a subject that is at high risk of being exposed to a pathogenic virus in order to prevent viral infection.

16. An antiviral composition in unit dosage form comprising an effective antiviral amount, between 1 and 250 mg, of a hydroxamic acid derivative or a physiologically acceptable salt thereof or a physiologically acceptable chelate thereof.

17. The antiviral composition of claim 16, which comprises between 2 and 100 mg of deferoxamine mesylate packaged in a sterile vial.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/02078

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/19
US CL :514/575
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS & CAS Online: hydroxamic acid, desferoxamine, viral, virus, antiviral, replication, reverse transcript?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Experientia, 15 February 1968, (GALE ET AL.), "Effects of Certain Hydroxamic Acids on Viral Replication", vol. 24, no. 2, pp. 194-195.	1-17
A	Cancer Research Effects of Deferoxamine on Human Myeloid Leukemia Cell Lines", vol. 49, pp. 4809-4812. 01 September 1989 (Becton et al.)	1-17
A	British Journal of Hematology, 1976,(Hoffbrand et al.,)"Effect of Iron Deficiency and Desferrioxamine on DNA Synthesis in Human Cells", vol. 33, pp. 517-525.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search	Date of mailing of the international search report
03 AUGUST 1992	03 SEP 1992
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/02078

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any addtional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention frist mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International application No.
PCT/US92/02078

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1-14, drawn to inhibiting viral growth with a hydroxamic acid derivative, classified in class 514, subclass 575.

Group III, claims 16-17, drawn to compositions of hydroxamic acid derivatives, classified in class 514, subclass 575.

Groups I-II are distinct from group III in that PCT Rule 13.1 does not provide for methods of using and composition within a single inventive concept. Group I is distinct from group II since they are drawn to distinct & distinct & separate methods.

Group II, claim 15, drawn to preventing viral replication with a hydroxamic acid derivative, classified in class 514, subclass 575.